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Original Research Article

Study of secondary hemo-phagocytic lympho-histiocytosis in children at a tertiary centre

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ABSTRACT

Background: Hemophagocytic lympho-histiocytosis (HLH) is fatal condition characterised by aggressive immune activation, dysregulation and up regulation of inflammatory cytokines leading to uncontrolled activation of T-cells, defective granule mediated cytotoxicity. HLH is classified into primary and secondary HLH.
Methods: This is retrospective study of case-records of children who fulfilled criteria of HLH-2004 guidelines from November 2016 to November 2020.
Results: Total number of patients with HLH was 33, excluding 4 cases of primary HLH, 29 cases met the inclusion criteria. 19 (65.5%) were children less than 5 years of age at the time of diagnosis. Six (20.7%) children were between 6 to 10 years and the rest 4 (13.8%) ten years. Average age at presentation was 4.93 years. Male to female ratio was 0.8 to 1. Fever was the chief complaint present in 29 (100%) of cases. Laboratory parameters showed anaemia, thrombocytopenia, hyper-ferritinemia and hypertriglyceridemia in all 29 (100%) cases. CRP elevated in 28 (96.6%) cases. Infections found in 29 (100%) of cases, bacteria in 19 (65.5%), viral in 10 (34.5%). Mortality seen in 11 (37.9%) cases. Average time of diagnosis from admission was 5.6 days. 4 children out of 11 had dengue as cause for secondary HLH and 4 had bacterial sepsis one each CMV, scrub typhus and SLE. Following factors affected the outcome by multivariate analysis, coagulopathy, elevated liver enzymes, bone marrow biopsy and immunosuppressive therapy.
Conclusions: HLH should be considered in differential diagnosis of children with sepsis. High index of suspicion for HLH with early initiation of treatment should be considered for better outcome.

Keywords: Infection, Secondary HLH, Children, Bacterial, Viral

INTRODUCTION

Hemophagocytic lympho-histiocytosis (HLH) is a fatal condition characterized by aggressive immune activation. It is a life-threatening condition characterized by immune dysregulation and up regulation of inflammatory cytokines that leads to uncontrolled activation of T-cells and defective granule mediated cytotoxicity.[1] HLH is classified into primary and secondary HLH. Primary or familial HLH is an inherited condition.[2] It manifests during infancy or early childhood. Exact incidence of secondary HLH data is not available. Familial HLH is an autosomal recessive condition due to defective immune regulation. It can be sporadic also at times. The PRF1, UNC13D, STX11 and STXBP2 are the genes commonly seen in primary HLH. It can also present in children with

immune deficiency conditions like Chediak Higashi syndrome, X-linked lymphoproliferative disease (XLP1 or XLP2), ITK deficiency and Griscelli syndrome. These immunodeficiencies are caused by genetic mutations.[3] Whereas secondary HLH is due to result of aggressive immune activation caused by infections which can be bacterial, viral or rarely parasitic and fungal infections. It is also reported secondary to autoimmune conditions like systemic lupus erythematosus and Kawasaki disease it also develops as a complication of malignancy and certain drugs. The diagnosis of HLH remains a challenge, as it is a rare disease with nonspecific signs and symptoms that mimic common conditions such as sepsis, systemic inflammatory response syndrome, and multiorgan dysfunction syndrome (MODS). Prior to HLH diagnostic criteria, children with prolonged fever were most often managed as fever of unknown origin. Now a child who meets the diagnostic criteria of the HLH-2004 protocol by the histiocyte society is managed with steroids and drugs like etoposide and cyclosporine to suppress the hyperinflammatory response.[4] HLH is diagnosed in the presence of a known HLH causing mutation or at least five out of the eight symptoms and signs are fulfilled. It is used for diagnosis of both primary and secondary HLH. I) Fever for more than 7 days, II) enlarged spleen (splenomegaly), III) Decrease in the number of blood cells (cytopenias), haemoglobin <9 gm/100 ml, platelets <100×103/ml, neutrophils <1×103/ml, IV) Elevated blood levels of triglyceride (hypertriglyceridemia fasting ≥265 mg/100 ml) and/or decreased blood levels fibrinogen (hypofibrinogenemia <150 mg/100 ml), V) Elevated blood levels of ferritin (hyperferritinemia) ≥500 µg/L, VI) Elevated blood levels of soluble CD25 (IL-2Ra) ≥2400 U/mL, VII) Decreased or absent NK cell killing activity, VIII) Presence of hemo-phagocytosis in bone marrow and/or cerebrospinal fluid. Once the diagnosis is made treatment is mainly focused on controlling the hyperinflammation. In primary HLH stem cell transplantation and to treat the underlying infection in secondary HLH is ideal. In many cases addition of steroids, intravenous immunoglobulins and sometimes chemotherapy with etoposide and cyclosporine is also required.[5] As mortality is very high from the time of diagnosis a high index of suspicion for HLH must be there and treatment should be initiated at the earliest.[4]

Need for the study

Exact incidence of secondary HLH is not available. However, from the available data familial HLH cases are less common than secondary HLH.[6] As the triggers involving infectious agents, are varied in a tropical country with geographic and seasonal variations.

METHODS

This is a retrospective study of case-records of all the children who were admitted at Indira Gandhi institute of child health, a tertiary care centre at Bangalore, and fulfilled the criteria of HLH- 2004 guidelines from November 2016 to November 2020. Institutional ethical committee clearance was obtained. Sample size was not calculated as it is a retrospective study and as the exact incidence of secondary HLH is uncertain. Children more than one month and less than eighteen years of age were included in this study. Familial cases of HLH were excluded from this study. Since the tests for soluble CD25 levels and NK cell activity were not available in our institute, diagnosis was made when five out of the other six criteria were fulfilled.

In study population, the following demographic data was analysed: age, sex, detailed physical examination findings, laboratory findings including complete blood count with differential [white blood cell (WBC), neutrophil, lymphocyte, and platelet count, concentrations of haemoglobin (Hb)], fibrinogen, coagulation function test [activated partial thromboplastin time (APTT), prothrombin time (PT)], and biochemical examinations [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, triglycerides, lactate dehydrogenase (LDH), serum ferritin (SF), C reactive protein (CRP)]. In addition, bone marrow aspiration findings, tests for collagen vascular disease, mycoplasma, Epstein-Barr virus (EBV), cytomegalovirus (CMV) and toxoplasma infection were diagnosed with immunoglobulin M antibodies. Other serological tests for infections like dengue, HIV, malaria, salmonella, brucella and rickettsia infection were recorded. Cultures (blood, urine and cerebrospinal fluid) and imaging findings (chest x-ray, abdominal ultrasound and computerized tomography) were recorded.

Treatment modalities (corticosteroid, immunochemotherapy consisting of cyclosporine, etoposide) and also supportive care (for opportunistic infection, coagulopathy, hepatic damage, and renal failure), and outcomes (survival rates, deaths) were analysed.

Statistical analysis was performed using SPSS software for windows. Descriptive analysis was expressed in arithmetic mean, percentage and median. Data was entered in Microsoft excel data sheet and analysed using SPSS 23 version software. Categorical data was represented as mean and standard deviation. Chi-square was used for categorical variables as the test of significance. Independent t test or Mann Whitney U test was used as the test of significance to identify the mean difference between two groups. Wilcoxon rank sum test and Kruskal Wallis test was used for skewed continuous variable. Univariate analysis and regression analysis was used to identify relationship between variables. The p value less than 0.05 was considered significant.

RESULTS

The total number of patients with HLH in the study period was 33. We have excluded four cases of primary

HLH involving familial HLH type 2, Chediak-Higashi syndrome, Griscelli syndrome and primary immunodeficiency cases (SCID). Remaining 29 cases who met the inclusion criteria were included in this study. 19 (65.5%) were children less than 5 years of age at the time of diagnosis. Six (20.7%) children were in the age group of 6 to 10 years and the rest 4 (13.8%) more than ten years. Male to female ratio was 0.8 to 1. Fever of more than seven days duration was the chief complaint present in all 29 (100%) of cases. Generalized lymphadenopathy was seen in 8 (27.6%). Abdominal distension was present in hundred percentage of cases due to organomegaly. Central nervous symptoms including seizures and altered sensorium was seen in 5 (17.2%) of these children. On examination of abdomen, hepatomegaly was seen in 29 (100%) and splenomegaly in 28 (96%).

Laboratory parameters showed anaemia, thrombocytopenia, hyper-ferritenemia and hyper-triglyceridemia in all 29 (100%) cases. CRP was elevated in 28 (96.6%) cases. Infection found in all 29 (100%) cases. Of which bacteria was seen in 19 (65.5%) and viral in 10 (34.5%) of them. Bacterial isolates included *Klebsiella* 3 (10.3%), MRSA 2 (6.9%), Acinetobacter 3 (10.3%), *E. coli* 2 (6.9%), tuberculosis 1 (3.4%), pseudomonas 2 (6.9%) and *Burkdofelia cepacia* 1 (3.4%) scrub typhus in 5 (17.2%), dengue was found in 7 (24.1%) cases, EBV in 2 (6.9%) and CMV in 1 (3.4%) case (Table 1).

Mortality was seen in 11 (37.9%) cases. Of which 5 (63.6%) were under 5 years of age, 3 (27.3%) were in the age group of 6 to 10 years and one child (9.1%) was older than 10 years. Average time of diagnosis from admission was 6.2 days. And average duration from diagnosis to death was 4.9 days. 4 children out of 11 had dengue as a cause for secondary HLH and 4 had bacterial sepsis 1 CMV, 1 Scrub typhus and 1 had SLE. Liver enzymes, hyperbilirubinemia, neutropenia and other blood investigations were not significant with respect to mortality. Following factors were found to affect the outcome by multivariate analysis, coagulopathy, elevated liver enzymes, bone marrow biopsy and immunosuppressive therapy. By regression analysis it was found only bone marrow biopsy was the factor which affected the outcome, p value was significant.

Table 1: Distribution of laboratory parameters.

Variables	N	Percentage (%)
Anemia	29	100
Thrombocytopenia	29	100
Neutropenia	5	17.2
CRP	28	96.6
Coagulopathy	14	48.3
Elevated liver enzymes	16	55.2
Hyperbilirubinemia	5	17.2
Hyper-ferritinemia	29	100
Hyper triglyceridemia	29	100
High LDH	23	79.3

Table 2: Organism distribution among subjects.

Variables		N	Percentage (%)
Etiology	Bacterial sepsis	14	48.3
	Viral	10	34.5
	Rickettsial infection	5	17.2
	Fungal	0	0.0
	Protozoal like malaria	0	0.0
Immunosuppression therapy	Not given	23	79.3
	Given	6	20.7
Outcome	Death	11	37.9
	Improved	18	62.1
Organisms	Acinetobacter	3	10.3
	Burkholderia cepacian	1	3.4
	CMV	1	3.4
	Dengue	7	24.1
	EBV	2	6.9
	E. coli	2	6.9
	Klebsiella sepsis	3	10.3
	MRSA sepsis	2	6.9
	Pseudomonas	2	6.9
	Scrub typhus	5	17.2
	Tuberculosis (associated with SLE)	1	3.4

Table 3: Association between parameters and outcome.

Variables		Outcome				P value
		Death		Improved		
		N	%	N	%	
Age (Years)	<5	7	63.6	12	66.7	0.717
	6 to 10	3	27.3	3	16.7	
	>10	1	9.1	3	16.7	
Sex	Female	6	54.5	10	55.6	0.958
	Male	5	45.5	8	44.4	
Fever	Abnormal	11	100	18	100	-
	Normal	0	0.0	0	0	
Lymphadenopathy	Abnormal	2	18.2	6	33.3	0.376
	Normal	9	81.8	12	66.7	
Respiratory symptoms	Abnormal	3	27.3	9	50	0.228
	Normal	8	72.7	9	50	
CNS symptoms	Abnormal	0	0	5	27.8	0.055
	Normal	11	100	13	72.2	
Hepatomegaly	Abnormal	11	100	18	100	-
	Normal	0	0	0	0	
Splenomegaly	Abnormal	11	100	17	94.4	0.426
	Normal	0	0	1	5.6	
Anemia	Abnormal	11	100	18	100	-
	Normal	0	0	0	0	
Thrombocytopenia	Abnormal	11	100	18	100	-
	Normal	0	0	0	0	
Neutropenia	Abnormal	1	9.1	4	22.2	0.364
	Normal	10	90.9	14	77.8	
CRP	Abnormal	10	90.9	18	100	0.193
	Normal	1	9.1	0	0	
Coagulopathy	Abnormal	9	81.8	5	27.8	0.005
	Normal	2	18.2	13	72.2	
Elevated liver enzymes	Abnormal	9	81.8	7	38.9	
	Normal	2	18.2	11	61.1	
Hyper-bilirubinemia	Abnormal	4	36.4	1	5.6	
	Normal	7	63.6	17	94.4	
Hyper-ferritinemia	Abnormal	11	100	18	100	-
	Normal	0	0	0	0	
Hyper triglyceridemia	Abnormal	11	100	18	100	-
	Normal	0	0	0	0	
High LDH	Abnormal	9	81.8	14	77.8	0.794
	Normal	2	18.2	4	22.2	
BM biopsy	Abnormal	1	9.1	13	72.2	0.002
	Normal BM study	2	18.2	0	0	
	BM study not done	8	72.7	5	27.8	
Etiology	Bacterial sepsis	5	45.5	9	50	0.514
	Viral	5	45.5	5	27.8	
	Rickettsia infection	1	9.1	4	22.2	
	Fungal	0	0	0	0	
	Protozoal like malaria	0	0	0	0	
	Autoimmune	0	0	0	0	
Immuno-suppression therapy	Not Given	11	100	12	66.7	0.032*
	Given	0	0	6	33.3	
IVIG	Not given	11	100	18	100	-
	Given	0	0	0	0	

DISCUSSION

HLH is a rare disorder that is being increasingly recognized in children now. Secondary HLH arises from infectious, rheumatologic, malignant or metabolic causes. To identify the underlying trigger, management of the uncontrolled immune activation is crucial. Despite the increase in the awareness of HLH, it is still difficult to differentiate it from other overlapping conditions. In our study the most common presentation was fever, hepatomegaly and splenomegaly. The most frequent laboratory findings were hyper-ferritinemia, hypertriglyceridemia and high CRP. Similar findings were reported in an earlier study. Hypertriglyceridemia was seen in 29 (100%) of our cases, unlike high triglycerides seen in only 70% of patients in Janka et al study.[6] BM biopsy done on 16 patients, hemo-phagocytosis detected in 14 (48.3%) patients. Unlike most of the previous studies.[7-9] in which viral etiology was more commonly associated with HLH, in our study, bacterial agents formed the majority among the pool of infectious diseases,19(65.5%) followed by viral agents,10 (34.5%). Among 26 cases of infection-associated hemo-phagocytic lympho-histiocytosis (IA-HLH) reported by Nair et al.[10] from all age groups, the majority of infections were bacterial (42%), followed by viral (35%). Simon et al also noticed a similar finding in their combined descriptive study analysing data of HLH patients that infectious triggers were seen in 62% (33) of cases of which bacterial (14) infections was followed by viral infections.[11]Another study of secondary HLH in 18 children in Turkey showed an equal incidence of bacterial and viral agents. Out of the 18 children in the age group of 2 weeks to 72 weeks 8 children had bacterial (44.4%) infections and eight children had viral (44.4%), while two (11.2%) of them had parasitic infestation.[12] The previous Indian studies by Raju et al.[13] have found dengue as the single most common infectious etiology associated with HLH. Raju et al study from south India on dengue patients showed that out of 212 confirmed cases of dengue, 31 patients had features suggestive of HLH in which 23 (10.84%) were confirmed by bone marrow. In a retrospective study by Bhattacharya et al following a dengue outbreak in 2017, 7 (32%) of dengue cases had HLH.[14] Viral infections were seen in 67% of study population in south India and dengue in 52% (27) cases.[15] Whereas scrub typhus was found in higher percentage as a trigger in northern India 27% by Parajuli et al it was seen in 17.7% of cases in our study.[16]

Of the 29 cases, 18 (41.3%) had resolution of disease with only supportive treatment. These were purely secondary HLH with mild disease activity. The current guidelines strongly recommend use of immunosuppressive agents in patients with severe secondary HLH. In our study immunosuppressive treatment was given for 20.7% cases with severe form of secondary HLH and all survived and found that immunosuppressive agents are statistically significant independent predictor of mortality. A study by Giri et al which analysed the use of the steroids as the sole modality in secondary HLH, showed that 83% of these cases survived.[17] The overall mortality is 11 (37.9%) in our study. Ramachandra et al reported lower mortality of 24%, which they attributed to higher incidence of secondary HLH, early diagnosis, and prompt treatment.[18] In our study coagulopathy, elevated liver enzymes, bone marrow biopsy and immunosuppressive therapy were found to affect the outcome. Age, clinical presentation, hemogram parameters, ferritin, triglyceride level did not influence the likelihood of death. Four of the 18 children who survived were lost to follow up in this study.

Of the 11 children who succumbed to death during hospital stay 4 (36.3%) of them had dengue and required ventilator and inotrope support. Five (45.45%) had bacterial sepsis including MRSA, pseudomonas, *E. coli*, one (9.09%) scrub typhus and one (9.09%) had CMV infection. Late referral and delay in early recognition of the condition and complications including shock, febrile neutropenia, multi organ disfunction lead to mortality.

Limitations of the study is genetic analysis and immunological investigations could not be done in all cases as it was a retrospective study.

CONCLUSION

High index of suspicion and early diagnosis of secondary HLH can improve the outcome. Our study emphasizes that bacterial and viral infections can trigger secondary HLH. Supportive treatment with immunosuppressive agents can improve the outcome. Further studies based on HLH registries at national level may help in better analysis and understanding of the condition.

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